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Influence of substrate concentration and feed frequency on ammonia inhibition in microbial fuel cells

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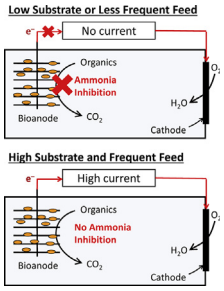
HIGHLIGHTS

- Ammonia inhibition was found to be dependent on substrate feed conditions in MFC.
- High substrates allow stable current generation at high ammonia concentration.
- Frequent substrate feed also makes bioanodes resistive against ammonia inhibition.
- Power density curves can predict ammonia inhibition before bioanodes are damaged.
- Continuously monitored current does not show inhibition until bioanodes are damaged.

GRAPHICAL ABSTRACT



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ABSTRACT

Excessive amounts of ammonia are known to inhibit exoelectrogenic activities in microbial fuel cells (MFCs). However, the threshold ammonia concentration that triggers toxic effects is not consistent among literature papers, indicating that ammonia inhibition can be affected by other operational factors. Here, we examined the effect of substrate concentration and feed frequency on the capacity of exoelectrogenic bacteria to resist against ammonia inhibition. The high substrate condition (2 g L^{-1} sodium acetate, 2-day feed) maintained high electricity generation (between 1.1 and 1.9 W m^{-2}) for total ammonia concentration up to 4000 mg-N L^{-1}. The less frequent feed condition (2 g L^{-1} sodium acetate, 6-day feed) and the low substrate condition (0.67 g L^{-1} sodium acetate, 2-day feed) resulted in substantial decreases in electricity generation at total ammonia concentration of 2500 and 3000 mg-N L^{-1}, respectively. It was determined that the power density curve serves as a better indicator than continuously monitored electric current for predicting ammonia inhibition in MFCs. The chemical oxygen demand (COD) removal gradually decreased at high ammonia concentration even without ammonia inhibition in electricity generation. The experimental results demonstrated that high substrate concentration and frequent feed substantially enhance the capacity of exoelectrogenic bacteria to resist against ammonia inhibition.

1. Introduction

Bioelectrochemical systems can be used to effectively treat wastewater and produce renewable energy. Microbial fuel cells (MFCs) are a type of bioelectrochemical system that can remove organics in wastewater and simultaneously produce electrical

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energy. Recent developments in MFC design (e.g., air cathodes [1]; sandwiched electrode assemblies [2]; inexpensive cathode catalysts [3]) along with pilot-scale demonstrations [4–7], make MFCs an attractive alternative for sustainable wastewater treatment and energy recovery [8]. Electric energy generation in MFCs relies on electrochemically active bacteria that form a biofilm on the anode. These exoelectrogenic bacteria oxidize organic substrates present in wastewater and transfer electrons to the anode, creating electric current. At the cathode of MFCs, water is formed through the reduction of oxygen that can be provided directly from the atmosphere using air cathodes [9]. The performance of MFCs as a wastewater treatment and energy recovery process centers on the activity of exoelectrogenic bacteria to transfer electrons to the anode; hence, the sensitivity of exoelectrogenic bacteria to various wastewater treatment conditions (e.g., ammonia, salinity, oxygen, etc.) needs to be investigated.

Recent studies have examined MFC performance with various sources of wastewater, including swine wastewater, anaerobic digester supernatant and human urine [10–12]. These high strength wastewaters contain excessive amounts of ammonia that can inhibit microbial metabolism and thus prevent exoelectrogenic bacteria from generating electric current in MFCs. In this study, we primarily focused on the effect of TAN (total ammonia nitrogen) on MFC performance under various substrate feed conditions.

Ammonia is well known for its cytotoxic effects on microorganisms [13,14]. Specifically, ammonia inhibition mechanisms include enzymatic activity disruption, alteration in the intracellular pH, and dehydration due to osmotic water loss [15–17]. Previous experimental studies consistently demonstrated negative responses of MFC bioanodes to significantly high TAN levels beyond 4000 mg-N L^{-1} [18–20]. However, the previous studies showed inconsistent results on the threshold TAN level that triggers ammonia inhibition effects in bioelectrochemical systems. For instance, Nam et al. reported that TAN concentrations exceeding 500 mg-N L^{-1} at neutral pH of 7 can result in severe inhibition of electricity generation in MFCs, implying that a relatively low ammonia concentration can trigger limited performance of bioanodes [18]. In another study using a continuously operated MFC, electric current generation gradually increased with increasing TAN concentrations up to 3500 mg-N L^{-1} [19]. Similarly, Kuntke et al. found no cytotoxic effects of ammonia up to 4000 mg-N L^{-1} [20]. It should be noted that experiments in these previous studies were performed under neutral pH conditions using phosphate buffer, indicating that equilibrium between free ammonia (NH_3) and ammonium ions (NH_4^+) did not affect the degree of ammonia inhibition as free ammonia is known to be more toxic to microorganisms than ammonium ions in biological wastewater treatment [14,21]. Thus, here we aim to explain the reported inconsistent TAN concentrations that trigger the ammonia inhibition effects in MFCs.

To further investigate the effects of ammonia inhibition in MFCs, we hypothesized that the substrate concentration and feed frequency can affect the capability of exoelectrogenic bacteria to generate electric current under high ammonia conditions. To our knowledge, none of the previous studies have investigated potential effects of the level and frequency of substrate feed on the ammonia inhibition effects in MFCs. MFC performance is often described with continually monitored current results as well as power density curves developed using various external resistors. Between the two performance indicators, we also investigated which can better predict ammonia inhibition before MFC bioanodes are completely damaged by high ammonia conditions.

2. Material and methods

2.1. MFC configuration and operation

Five single-chamber MFCs were constructed using polypropylene blocks with an inner cylindrical chamber (23 mL; 7 cm^2 in cross section). Graphite fiber brushes (2 cm diameter and 2.5 cm in length; Mill-Rose, OH) were pretreated in a muffle furnace at 450 °C for 30 min [22] and used as the bioanode. The bioanodes were inoculated with primary clarifier effluent and digested sludge collected from a domestic wastewater treatment plant. All MFCs underwent an enrichment period of about 5 months. The air cathodes (7 cm^2 in cross section) were prepared using wet proofed carbon cloth (Fuel Cell Earth, MA) with a Pt/C catalyst (0.5 mg cm^{-2}), as previously described [1]. Constructed MFCs were operated under fed-batch mode with an external resistance (100 Ω).

To study the inhibitory effect of ammonia at different substrate levels and feed frequencies, two MFCs were fed with 2 g L^{-1} sodium acetate every two days (high acetate and short cycle, HASC-1 and -2); one with 0.67 g L^{-1} sodium acetate every two days (low acetate and short cycle, LASC); and two with 2 g L^{-1} sodium acetate every 6 days (high acetate and long cycle, HALC-1 and -2) (Table 1). The feed medium was prepared with sodium acetate (according to Table 1) in 50 mM phosphate buffer solution (4.7 g L^{-1} Na_2HPO_4; 0.6 g L^{-1} $NH_4H_2PO_4$; 1.6 g L^{-1} KH_2PO_4; 0.4 g L^{-1} $NaHCO_3$), and trace amounts of vitamins and minerals [23]. The total amount of ammonia was adjusted by adding NH_4Cl in the feed medium and was gradually increased from 100 to 4000 mg-N L^{-1}. For each ammonia condition, MFCs were operated for 6 days (i.e., 3 short fed-batch cycles or one long fed-batch cycle) except for the very high ammonia concentrations (3500 and 4000 mg-N L^{-1}). At the end of each fed-batch cycle, the solution in the MFCs was completely removed from the reactor and the reactor was refilled with the fresh medium solution.

2.2. Experimental measurements

Electric current in the reactors was determined by measuring the voltage drop every 20 min across an external resistor of 100 Ω using a multimeter and data acquisition system (Model 2700, Keithley Instruments, OH). After each fed-batch cycle, effluent was analyzed for conductivity and pH (SevenMulti; Mettler-Toledo International Inc., OH). The fresh medium pH was stable due to the sufficient phosphate buffer capacity (50 mM), slightly decreasing from 7.1 to 6.7 with increasing TAN concentration. The effluent from the MFC was also neutral between pH 6 and 7 for the TAN concentration 500 mg-N L^{-1} or higher. This stable pH condition indicates that the speciation between free ammonia (NH_3) and ammonium ion (NH_4^+) was kept constant during the experiment. The chemical oxygen demand (COD) was determined according to standard methods (Hach Co., CO) [24]. All experiments were performed in an air-conditioned laboratory and temperature was stationary over the course of MFC operation at 23.2 ± 0.8 °C. Note that temperature affects the ammonia speciation between free ammonia and ammonium ion. For instance, the amount of free

Table 1
Three different operation conditions in fed-batch experiments.

MFC operation condition	Substrate level (g L^{-1} sodium acetate)	Batch cycle length (days)
HASC (high acetate, short cycle)	2.0	2
LASC (low acetate, short cycle)	0.67	2
HALC (high acetate, long cycle)	2.0	6

ammonia at 23.2 °C is about 62% of that at 30 °C for a given TAN concentration [18].

To prepare power density curves, the MFC electrodes were disconnected (open circuit) for 60 min and then serially connected to an external resistor of 10, 100, 200, 400 and 1000 Ω every 10 min. The power density (P) was calculated from $P = VI$, where V is the measured voltage drop across the external resistor, I is the current density normalized by the projected surface area of the cathode (7 cm^2). This power density test was performed on the last day of an applied ammonia condition.

2.3. Coulombic efficiency

The Coulombic efficiency (CE) is the ratio between the measured electron generation and theoretical amount of electron production by substrate oxidation as [8]:

$$CE = \frac{8\int i dt}{FV_{MFC}\Delta COD} \quad (1)$$

F is the Faraday's constant (96,485 C mol^{-1}), i is the electric current, ΔCOD is the change in COD over a fed-batch cycle and V_{MFC} is the volume of the MFC reactor.

3. Results and discussion

3.1. Inhibition of current generation

The exoelectrogenic microorganisms were capable of generating current at a maximum capacity (~3.8 mA) for all MFC reactors at TAN concentration up to 2500 mg-N L^{-1} (Fig. 1). The current results for TAN concentration from 100 to 2000 (not shown) exhibited increases from 2.5 to 3.8 mA due to the conductivity increase in the solution. The effect of conductivity on reactor performance has been demonstrated previously [18,19]. HASC-1 and -2 maintained high current generation for the high TAN concentration up to 4000 mg-N L^{-1} (~3.5 mA) (Fig. 1(A)). Some variability existed between HASC-1 and -2, particularly at the start of the high ammonia conditions (i.e., 3500 and 4000 mg-N L^{-1}). HASC-2 showed consistently high current generation while HASC-1 experienced a substantial decrease in current generation for the first few fed-batch cycles under 3500 and 4000 mg-N L^{-1}. However, the current generation in HASC-1 was rapidly restored in ~5 days to comparable levels of that in HASC-2.

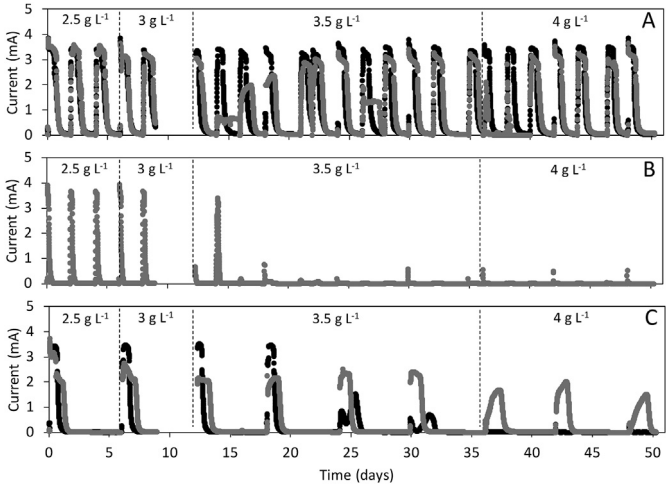

Fig. 1. Effects of ammonia concentration on current generation in single-chamber MFCs. (A) HASC-1 (●), HASC-2 (●) (every 2-day feed – 2 g L^{-1} CH_3COONa); (B) LASC-1 (●) (every 2-day feed – 0.67 g L^{-1} CH_3COONa); and (C) HALC-1 (●), HALC-2 (●) (every 6-day feed – 2 g L^{-1} CH_3COOAc). (Lost data between days 9 and 12).

The low substrate condition (0.67 g L^{-1} sodium acetate) resulted in immediate impairment of current generation at 3500 mg-N L^{-1} for LASC-1 (Fig. 1(B)). In comparison with the current generation from HASC (2 g L^{-1} sodium acetate) (Fig. 1(A)), it is concluded that a certain level of substrate concentration should be provided to keep exoelectrogenic bacteria active under high ammonia conditions.

The frequency of substrate feed was also an important factor that determines the capability of exoelectrogenic bacteria to resist against high ammonia concentration. The HALC MFCs (fed with 2 g L^{-1} sodium acetate, but less frequently every 6 days) showed gradual decreases in current generation at 3500 mg-N L^{-1} (Fig. 1(C)). HALC-1 exhibited a reduction in current by ~20% at 3000 mg-N L^{-1} and a further decrease at 3500 mg-N L^{-1}, but remained consistent across the four batch cycles at this ammonia level. HALC-2 exhibited no decreases in current until the third batch cycle at 3500 mg-N L^{-1}, but showed a rapid drop in current generation thereafter. While HALC-1 and -2 responded differently to the high ammonia conditions, current generation in both MFCs was substantially inhibited by the high ammonia concentration. This result compared to the consistently high current generation in HASC-1 and -2 (fed every 2-days) clearly demonstrates that the capacity of exoelectrogenic bacteria to resist against high ammonia concentration is substantially enhanced by feeding MFCs frequently.

Since the oxidation of acetate by exoelectrogenic bacteria creates protons (Eq. (2)), the high substrate concentration and frequent feed conditions may have provided continuously low local pH conditions near the anode surface. As a result, low pH conditions make ammonium ion (NH_4^+) dominant over free ammonia (NH_3), which is known to be more toxic than ammonium ion.

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad (2)$$

Ammonia losses over a fed-batch cycle were observed due to active nitrification only at relatively low ammonia concentration conditions (500–2000 mg-N L^{-1}). It should be emphasized that the current generation was active for these TAN concentrations, indicating that nitrate or nitrite, which is also known to inhibit microbial activities at high concentration, did not affect the exoelectrogenic activity. In addition, at TAN of 3500 and 4000 mg-N L^{-1} where the high ammonia level started inhibiting current generation in HALC and LASC, nitrification was substantially limited due to the high ammonia concentration as previously reported [18]. These observations indicate that nitrification and resulting nitrate or nitrite hardly affected the MFC performance in the experiment.

3.2. Ammonia inhibition on power generation

HASC-1 and -2 (2 g L^{-1} sodium acetate and 2-day feed) maintained high maximum power densities between 1.1 and 1.9 W m^{-2} (35 and 59 W m^{-3}) for all TAN concentrations up to 4000 mg-N L^{-1} (Fig. 2). The maximum power density was high between 1.4 and 2.0 W m^{-2} (45 and 62 W m^{-3}) for LASC-1 only at TAN concentrations of 3000 mg-N L^{-1} or less (Fig. 2(A), (B), (C) and (D)), indicating the low substrate concentration (0.67 g L^{-1} sodium acetate) did not limit the electric power generation. However, at 3500 and 4000 mg-N L^{-1}, the maximum power density decreased to 7.1×10^{-8} and 1.7×10^{-6} W m^{-2} (2.2×10^{-6} and 5.4×10^{-5} W m^{-3}), respectively (Fig. 2(E) and (F)). The maximum power density for HALC-1 and -2 showed a gradual decrease from 2.3 to 0.6 W m^{-2} (72–17 W m^{-3}) (HALC-1) and from 1.1 to 0.9 W m^{-2} (35–28 W m^{-3}) (HALC-2) as the

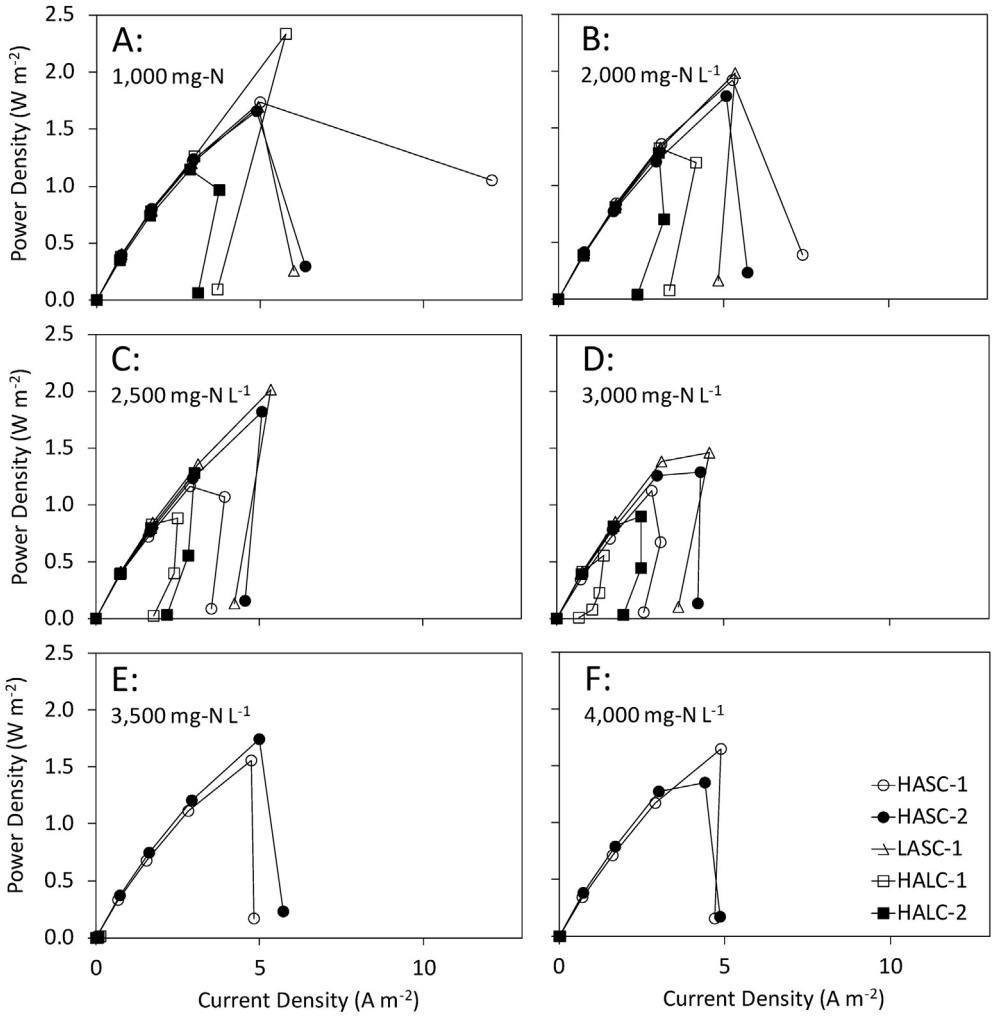

Fig. 2. Power density curves for total ammonia concentration of (A) 1000 mg-N L^{-1}, (B) 2000 mg-N L^{-1}, (C) 2500 mg-N L^{-1}, (D) 3000 mg-N L^{-1}, (E) 3500 mg-N L^{-1}, and (F) 4000 mg-N L^{-1}.

TAN concentration increased from 1000 to 3000 mg-N L^{-1}. It should be noted that the maximum power density of HALC-1 was the highest (2.3 W m^{-2}) among the 5 MFCs at TAN of 1000 mg-N L^{-1}, indicating that the less frequent fed-batch operation (every 6 days) did not limit the power generation at the relatively low TAN concentration (1000 mg-N L^{-1}). The high TAN concentration (4000 mg-N L^{-1}) resulted in substantial reduction in the maximum power density to 4.7×10^{-4} and 5.0×10^{-5} W m^{-2} (1.4×10^{-2} and 1.5×10^{-3} W m^{-3}) for HALC-1 and -2, respectively. The power density results confirmed that a sufficient amount of substrates coupled with frequent feed keeps exoelectrogenic bacteria robust against high ammonia concentrations at 3000 mg-N L^{-1} or higher in MFCs.

The current generation and power density results consistently showed that the HASC reactors outperformed the LASC and HALC reactors under relatively high ammonia concentration conditions (>3000 mg-N L^{-1}). The higher organic substrate level (2 g L^{-1} sodium acetate) and frequent fed-batch cycle (every 2 days) negated the inhibitory effects of ammonia that influenced the other reactors. Even though HASC-1 showed a sudden drop in current generation when the ammonia concentration was increased to 3500 and 4000 mg-N L^{-1}, the current was rapidly restored in 2 or 3 fed-batch cycles (Fig. 1(A)). Neither the LASC nor HALC reactors were able to recover from the decreased current, indicating permanent damages on the bioanode by high ammonia concentration. As a result, the power production was practically zero when the LASC and HALC reactors were examined in the polarization experiments. Note that the greater current and power generation in HASC (every 2-d feed) than those in HALC (every 6-d feed) indicates that there were no potential cathode catalyst losses.

It was found that the power density curve serves as a better indicator for predicting ammonia inhibition in bioelectrochemical systems than continuously monitored electric current. As the ammonia concentration gradually increased up to 3000 mg L^{-1}, the electric current was consistently high (>3 mA) for all of the five MFCs (Fig. 1) without showing any inhibition effects. However, the MFCs with either the low initial substrate or long fed-batch cycle condition (HALC-1, HALC-2 and LASC-1) showed clear decreases in the maximum power generation at the ammonia concentration of 3000 mg-N L^{-1} (Fig. 2(D)). In addition, HALC-1 maintained a non-zero electric current (1–2 mA in Fig. 1(C)) under the high ammonia concentration of 3500 and 4000 mg-N L^{-1}; however, the power generation in HALC-1 was practically zero (Fig. 2(E) and (F)), indicating evident inhibition by the high ammonia concentration. Based on these experimental observations, we suggest that the ammonia inhibition in MFCs be determined using power density curves rather than monitoring electric current.

3.3. Effect of ammonia on COD removal in MFCs

The increasing ammonia level decreased the COD removal in MFCs (Fig. 3) as previously demonstrated [18]. The COD removal was almost complete (>90%) and consistent up to 1000 mg-N L^{-1}. However, the removal started to gradually decrease for all MFCs with the increasing ammonia concentration beyond 1000 mg-N L^{-1}. The MFCs with the high initial substrate concentration (2 g L^{-1} sodium acetate in HASC and HALC) showed a similar COD removal trend with the increasing ammonia concentration up to 4000 mg-N L^{-1}, decreasing from 98% COD removal to 72%. The MFC with the low initial substrate concentration (LASC) exhibited a more drastic decrease in COD removal from 97 to 49% with the increasing ammonia concentration.

3.4. Coulombic efficiency

The Coulombic efficiency (CE) without ammonia inhibition at the relatively low TAN concentrations was approximately 70% (Fig. 4). The Coulombic efficiency for HASC was consistently high (>50%) throughout the experiment because any ammonia inhibition effects were not observed due to the high initial substrate concentration (2 g L^{-1} sodium acetate) and frequent fed-batch operation (every 2 days). HALC (2 g L^{-1} sodium acetate and 6 day fed-batch operation) showed high CE ~70% for ammonia concentrations up to 3000 mg-N L^{-1}. The high ammonia concentration (3500 and 4000 mg-N L^{-1}) significantly decreased the Coulombic efficiency down to 27% (Fig. 4). This decrease in CE is consistent with the drop-off in current generation in the HALC reactor (Fig. 1(C)). The Coulombic efficiency for LASC decreased to 52% at 2500 mg-N L^{-1} and then further dropped down to 2.6% at 3500 mg-N L^{-1}. This substantial drop in CE can be explained by the negligible current generation at the ammonia concentration of 3500 and 4000 mg-N L^{-1} (Fig. 1(B)).

Even with the Coulombic efficiency below 3% at the high TAN concentrations (3500 and 4000 mg-N L^{-1}) (Fig. 4), LASC showed approximately 50–60% COD removal from the initial sodium acetate concentration of 0.67 g L^{-1} (Fig. 3). This percent COD removal indicates that approximately 0.3–0.4 g L^{-1} sodium acetate was consumed for 2 days by non-exoelectrogenic metabolisms (e.g., aerobic microorganisms in biofilms on the air cathode). For the HASC results at 3500 and 4000 mg-N L^{-1}, the CE was 50–60% (Fig. 4) while the COD removal was ~80% from the initial sodium acetate concentration of 2 g L^{-1} (Fig. 3). Thus, approximately 0.7 g L^{-1} of sodium acetate was consumed for 2 days by non-exoelectrogenic metabolisms, indicating that substrate consumption by non-exoelectrogenic microorganisms is ubiquitous regardless of the ammonia inhibition effect on exoelectrogenic bacteria. Note that the gradual decrease in the COD removal and CE (Figs. 3 and 4) can be explained by slight decreases in current generation in HASC with the increasing TAN concentration even though the peak current was kept high (Fig. 1(A)). This observation confirms that continuously monitored electric current is not sensitive enough to detect the ammonia inhibition in MFCs.

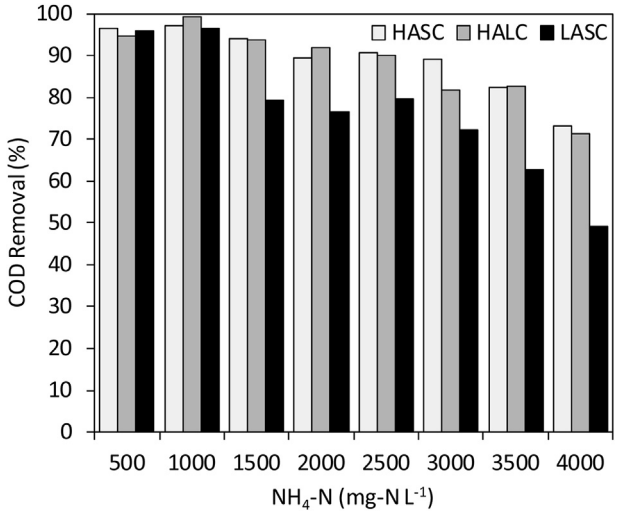

Fig. 3. COD removal results with gradually increasing total ammonia concentration.

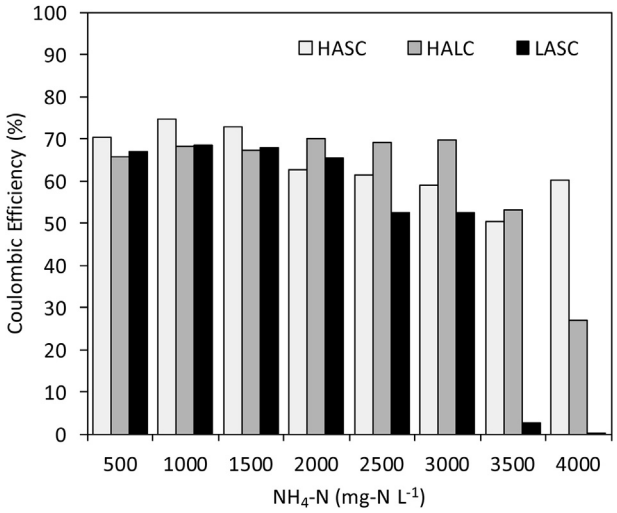

Fig. 4. Coulombic efficiency results with gradually increasing total ammonia concentration.

4. Conclusions

The link between the level of substrate concentration and ammonia inhibition in single-chamber MFCs was investigated. The low substrate condition (0.67 g L^{-1} sodium acetate) resulted in substantial impairment of current generation at 3500 mg-N L^{-1}. However, under the high substrate condition (2 g L^{-1} sodium acetate, every 2-day feed), high current generation was maintained for all TAN concentrations tested (up to 4000 mg-N L^{-1}). The result clearly indicates that a certain level of substrate concentration should be provided to keep exoelectrogenic bacteria active under high ammonia conditions.

The frequency of substrate feed (i.e., length of fed-batch cycle) was also found to be an important factor as the short fed-batch cycle (2 days) substantially enhanced the capability of exoelectrogenic bacteria to resist against ammonia cytotoxicity. The MFCs fed less frequently (every 6 days) started showing limited current generation at 3500 mg-N L^{-1} and low power generation at 2500 mg-N L^{-1}.

It was determined that the power density curve serves as a better indicator than continuously monitored electric current for predicting ammonia inhibition in bioelectrochemical systems. Although the power density curves and current generation results showed consistent responses to the increasing ammonia concentration, the ammonia inhibition effect was reflected early in the power density curves before the bioanode was completely damaged by excessive amounts of ammonia. It is suggested that ammonia inhibition be determined using power density curves rather than continuously monitoring electric current in MFCs.

The COD removal decreased with the increasing ammonia concentration. MFCs with the high substrate concentration (HASC and HALC) showed a similar COD removal trend with increasing ammonia concentrations while the low substrate concentration (LASC) exhibited a more drastic decrease in COD removal. The Coulombic efficiency was consistently high for the high substrate and frequent feed MFCs (HASC). The reactors with the high substrate and less frequent feeding (HALC) experienced lower Coulombic efficiencies for ammonia concentrations of 3500 and 4000 mg-N L^{-1}. The CE for the low substrate MFCs (LASC) started decreasing at an ammonia concentration of 2500 mg-N L^{-1} and substantially dropped down to less than 5% at 3500 mg-N L^{-1}. These CE results are consistent with the continuously monitored current generation.

The high current and power generation for the high substrate concentration (2 g L^{-1} sodium acetate) and frequent substrate feed (2 day fed-batch cycles) clearly demonstrated that the capacity of exoelectrogenic bacteria to resist against high ammonia concentration is substantially enhanced by keeping the substrate concentration high in MFCs. This conclusion along with findings in the previous studies [18–20] will allow reliable MFC applications for energy recovery and treatment of agricultural wastewater and source-separated human urine that contain excessive amounts of ammonia.

This study was performed with acetate as the sole substrate in MFCs, implying that the findings are applicable for bioanodes occupied mainly by *Geobacter* species. We suggest that future study examine the ammonia resistance with other substrates (e.g., glucose or lactate) or real wastewaters to induce other types of exoelectrogens to derive generalized conclusions on the correlation between the ammonia resistance and substrate feed condition.

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